Ecological Modelling 383 (2018) 160–170

Contents lists available at ScienceDirect

Ecological Modelling

journal homepage: www.elsevier.com/locate/ecolmodel

Modeling the influence of initial density and copper exposure on the interspecific competition of two algal species

Yongeun Kim[a], Jino Son[b], Hyoung-Ho Mo[c], Yun-Sik Lee[b], Kijong Cho[d,*]

[a] Institute of Environment and Ecology, OJERI, Korea University, Seoul 02841, Republic of Korea
[b] Ojeong Eco-Resilience Institute, Korea University, Seoul 02841, Republic of Korea
[c] Jungbu Regional Office, Animal and Plant Quarantine Agency, Incheon 22133, Republic of Korea
[d] Department of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, Republic of Korea

ARTICLE INFO

Keywords:
Allelopathy
Competitive dominance
Competitive response
Pseudokirchneriella subcapitata
Chlorella vulgaris

ABSTRACT

The interspecific competition among algal species is an important process that can change the community structure in aquatic ecosystems. However, there is still a lack of understanding of the impact of various factors on interspecific competition. In this study, both experimental and mathematical modeling approaches were employed to investigate how various combinations of the initial cell densities of *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* and copper exposure levels affect interspecific competition between these species. In the simulation results, *C. vulgaris* appeared to be superior to *P. subcapitata* in the absence of copper exposure. However, in the copper-exposed groups, the competitive positions of both algal species varied with the initial cell density and the copper exposure level. In particular, at the highest copper concentration (10 μg/L), *C. vulgaris* became less competitive than *P. subcapitata* in most initial cell density combinations, resulting in a shift in competitive dominance. This study clearly showed that the dominant species in the interspecific competition could be altered by the two factors studied herein. The developed model provided a more detailed and intuitive understanding of the effects of the two factors on the interspecific competition by simulating the competition at various combinations of initial algal density and copper exposure levels. In this study, the initial algal density and copper exposure levels were selected as the factors influencing the interspecific competition between *P. subcapitata* and *C. vulgaris*, but the proposed model could be used to study the effects of other toxicants on the interspecific competition between other algal species.

1. Introduction

Interspecific competition is one of the fundamental processes determining community structure (Chase et al., 2002), which in turn alters ecosystem function. In particular, an interspecific competition is observed more frequently in aquatic ecosystems than in terrestrial ecosystems (Connell, 1983), and the interactions among prey are known to have a significant impact on high-level predator populations (Garvey et al., 1994). Since algae play a crucial ecological role in freshwater ecosystems as the basis of many food chains and primary producers (Stoiber et al., 2012), it is important to understand interspecific competition among algal species. Intensified interspecific competition between algal species can lead to reduced growth of individuals, resulting in succession and shifts in algal community structure. Furthermore, the impacts of algal competition are likely to cascade through trophic levels (Granéli et al., 2008), which in turn eventually alter the biodiversity and community structure of freshwater ecosystems.

Several studies have demonstrated that various factors, such as allelochemicals (Hulot and Huisman, 2004), nutrition (Chakraborty et al., 2008), initial algal cell density (Tameishi et al., 2009; Qiu et al., 2011), predators (Carusela et al., 2009), and toxicants (Lürling and Roessink, 2006), are involved in algal species competition. Among these factors, allelochemicals, which are the chemical substances released by the algal species for inhibiting the growth of the other algal species, and initial cell densities are the most widely studied. Several algal species can have competitive dominance over others by producing allelochemicals (reviewed by Granéli et al., 2008). Tameishi et al. (2009) and Qiu et al. (2011) reported that the initial densities had a significant impact on the interspecific competition between *Prorocentrum minimum/Skeletonema costatum* and *Chattonella antiqua/Akashiwo sanguinea*, respectively, but Kuwata and Miyazaki (2000) reported no significant effect of the initial densities of *Microcystis novacekii* and *Scenedesmus quadricauda* on interspecific competition. Although

* Corresponding author.
E-mail address: kjcho@korea.ac.kr (K. Cho).

https://doi.org/10.1016/j.ecolmodel.2018.04.018
Received 19 December 2017; Received in revised form 24 April 2018; Accepted 27 April 2018

conflicting results have been reported in the literature, the initial algal cell density can have a significant direct or indirect impact on algal competition as it is linked to the quantity of the allelochemicals produced and preoccupancy/exploitation of limited nutrients (Hulot and Huisman, 2004). Another important factor is the presence of exogenous toxic substances. Algal populations are frequently exposed to various toxicants in the environment, and are one of the most toxicant-sensitive biota among aquatic organisms (Stoiber et al., 2012). Thus, because different algal species have different sensitivities, the dominant species in an aquatic ecosystem could change through the replacement of the sensitive species by the more resistant one (Lürling and Roessink, 2006). Toxicants could be an even more important factor than the other factors involved in algal species competition because they have adverse effects on both species simultaneously. Although the above-mentioned factors might affect the interspecific competition among algae species in a complex manner, no studies have considered the combined effects of these factors.

Since there are limits to experimentally observing the effects of various combinations of factors on the interspecific competition between algal species, a mathematical model can be a useful tool. However, to date, no mathematical model has taken into account the influences of both initial algal densities and toxicants on algal competition. The model proposed by Uchida et al. (1999) has been widely used to study the effects of initial algal density on interspecific competition (Yamasaki et al., 2007; Tameishi et al., 2009; Qiu et al., 2011). However, it is difficult to include the effects of other factors because the interactions between two algal species are simply represented by a single parameter as an interaction rate. On the other hand, the model proposed by Fergola et al. (2007) describes an allelopathic interaction in more detail by adding extra information on how an allelochemical concentration affects the interspecific competition, but no other factors were considered (DellaGreca et al., 2010). In addition, several models have been used to study the effects of specific factors on algal competition (*e.g.*, nutrient limitation: Chakraborty et al., 2008; predators: Carusela et al., 2009), but these models cannot be used to study the influence of initial algal density and toxicants on interspecific competition. Since the structure of a mathematical model depends on the research subjects, a new modeling approach is necessary to improve the understanding of how the combination of initial algal density and toxicants affect algal competition.

Therefore, in this study, a new mathematical model was proposed, and the influence of initial algal density and toxicant on interspecific competition was evaluated. Two freshwater algal species, *Pseudokirchneriella subcapitata* (formerly known as *Rhaphidocelis subcapitata* and *Selenastrum capricornutum*) and *Chlorella vulgaris,* were selected as model species because of their ecological relevance and availability (Silva et al., 2009; Machado et al., 2015). The production of an allelopathic substance (called chlorellin) by *C. vulgaris* makes it ideal for investigating the allelopathic competitive interactions (Fergola et al., 2007). Copper was chosen as a toxic substance because of its frequent detection in aquatic environments and high toxicity to various algal species (Flemming and Trevors, 1989).

The objective of this study was to investigate how various combinations of initial cell densities of two algal species, *P. subcapitata* and *C. vulgaris*, and copper exposure affect interspecific competition in a closed system through both experimental and mathematical modeling approaches. The conceptual diagram for interspecific competition between the two algal species and the flowchart of the study processes are shown in Fig. 1. To obtain the data sets for model calibration, various combinations of initial cell densities of both algal species were monitored periodically over time under copper exposure conditions (0, 5, and 10 μg/L). A mathematical model, including the effects of initial density, copper exposure levels, and allelopathy, was developed in order to explore their combined effects on the interspecific competition between the two algal species. Using the experimental data sets, the model was calibrated to predict the density dynamics of the two algal species over time, and the interspecific competition between the two species was evaluated in terms of competitive dominance, competitive response, and time required to reach the maximum algal density.

2. Experiments

2.1. Test algae and culture conditions

Two freshwater microalgal species *P. subcapitata* (strains CCAP 278/4) and *C. vulgaris* (strains AG40003) were obtained from the Culture Collection of Algae and Protozoa (CCAP, Scottish Marine Institute, UK) and the Korean Collection for Type Cultures (KCTC, Korea Research Institute of Bioscience and Biotechnology, Korea), respectively. The growth medium was prepared in accordance with the United Stated Environment Protection Agency (EPA) method 1003.0 (EPA, 2002). Both algal species were maintained in separate 200-mL Erlenmeyer flasks containing 100 mL of synthetic algal medium (EPA, 2002). The flasks were incubated at 20 ± 1 °C with a 16-h light/8-h dark photoperiod under illumination at 70 μmol photons/m^2/s with cool white fluorescent light (TLD 30 W, Philips). The maximum densities of *P. subcapitata* and *C. vulgaris* were approximately 4.0×10^6 and 4.0×10^7 cells/mL, respectively, under these culture conditions. The flasks were hand-agitated twice daily to minimize flocculation and clumping of algal cells. All glassware used in the algal culture maintenance and experiments were completely immersed in 10% nitric acid for at least 1 d and was thoroughly rinsed with distilled water before use.

2.2. Experimental designs

The copper stock solution was prepared by dissolving the reagent-grade copper (II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$, ≥99% purity, Sigma-Aldrich) in the synthetic algal medium. The stock solution was diluted with the synthetic medium to make the final copper concentrations of 5 and 10 μg/L. These copper concentrations were selected based on our previous study (unpublished data) in which the growths of *P. subcapitata* and *C. vulgaris* were severely inhibited when exposed to 15 μg/L or higher concentrations of copper. In addition, Franklin et al. (2002) reported that the 72 h EC_{50} values of copper for the growth inhibition of *P. subcapitata* and *Chlorella* sp. were 17 and 16 μg/L, respectively. The pH values of the test solutions were adjusted with 0.1 M HCl or 0.1 M NaOH to remain in the range of 7.5 ± 0.1.

To investigate how various initial cell density combinations of *P. subcapitata* and *C. vulgaris* affect the interspecific competition in either the presence (treatments) or absence of copper exposure (control), algal growth experiments were conducted in 100-mL glass beakers filled with 70 mL medium with several combinations of initial cell densities. To adjust the initial cell density of each species, both algal species were harvested at steady growth phase by centrifuging at 600 × *g* for 5 min and then resuspended in the growth medium. The initial cell densities of *P. subcapitata* and *C. vulgaris* were adjusted to range from 3.0×10^4 to 3.5×10^5 cells/mL and 2.0×10^5 to 4.0×10^6 cells/mL, respectively (Table 1). The combinations of initial cell densities were determined to include various *P. subcapitata*/*C. vulgaris* ratios in the estimation of model parameters. As reported previously (Hu and Zhang, 1993; Franklin et al., 2002), the initial cell density was set to a relatively high value in adverse conditions (*i.e.*, copper exposure). In each combination, five replicates were performed.

During the experiments, all beakers were sealed with Parafilm (polyethylene) to prevent the evaporation of the test medium, and were kept at the same environmental conditions as those used for culture maintenance. The beakers were shaken by hand twice a day. The algal cell densities were checked periodically (1–16-d interval) over the 78 days until the algal cell density in each beaker was close to zero. The number of algal cells in each combination was counted using a hemocytometer (Marienfeld, Germany) under an optical microscope (E200;

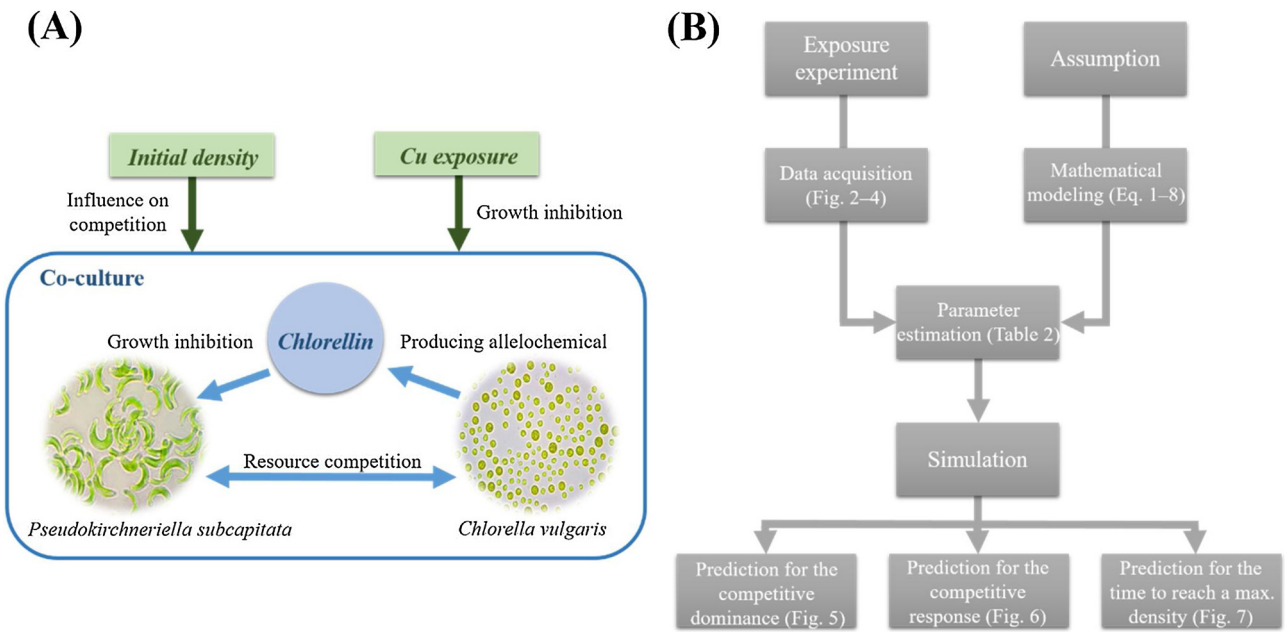

Fig. 1. Conceptual diagram of the interspecific competition between *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* affected by initial algal density and copper exposure (A) and the flowchart of the process used in this study (B).

Nikon, Japan) by taking 5 μL of algal suspension from each test beaker (four repeated measurements for each beaker). No additional process was applied to distinguish the species because of the apparent differences in shape and size between the two algal species (*P. subcapitata*: curved and twisted shape, 8–14 μm in length; *C. vulgaris*: spherical shape, 5–9 μm in diameter).

3. Model formulation

3.1. Generic assumption

A mechanistic model that describes the population dynamics of each algal species consists of two phases—growth and decline. It is assumed that the habitat quality (S), the capacity of an environment to provide conditions appropriate for individual and population persistence, is continuously reduced by a proportion to the population cell densities and growth rates at a given time in the given closed co-culture. As the cell density increases, the nutrients in the beaker continue to decrease and the toxic wastes continue to accumulate, subsequently degrading habitat quality. Thus, S, scaled to lie within a range of 0–1, reflecting habitat quality (0: fully degraded, 1: initial condition) is given as follows:

$$\frac{dS}{dt} = -m_{ps}X - \frac{dX}{dt}n_{ps} - m_{cv}Y - \frac{dY}{dt}n_{cv}, \quad (1)$$

where X and Y are the cell densities (cells/mL), m_{ps} and m_{cv} are the habitat depletion rates (mL/cells) of the cell maintenance, n_{ps} and n_{cv} are the habitat depletion rates (mL/cells) of the growth of *P. subcapitata* and *C. vulgaris*, respectively. Therefore, an algal population continues growing when S is greater than zero (growth phase) and goes to extinction (decline phase) when S reaches zero.

3.2. Growth phase

The growth phases of both algal species in the given closed co-culture can be described as follows:

$$\frac{dX}{dt} = \mu_{ps}SX - d_{ps}X$$
$$\frac{dY}{dx} = \mu_{cv}SY - d_{cv}Y, \quad (2)$$

where μ_{ps} and μ_{cv} are the specific growth rates (1/d) and d_{ps} and d_{cv} are the mortalities (1/d) of *P. subcapitata* and *C. vulgaris*, respectively.

Since *C. vulgaris* can produce allelochemicals (chlorellin) that inhibit the growth of both *C. vulgaris* and *P. subcapitata*, the growth rate of each species can be expressed by incorporating the chlorellin concentration-dependent growth rate (growth rate·$exp(-rp)$) into Eq. (2) (Fergola et al., 2007). Here, r is the inhibition constant and p is chlorellin concentration. Therefore, Eq. (2) can be rearranged as follows:

$$\frac{dX}{dt} = \mu_{ps}\exp(-r_{ps}p)SX - d_{ps}X$$
$$\frac{dY}{dx} = \mu_{cv}\exp(-r_{cv}p)SY - d_{cv}Y, \quad (3)$$

where r_{ps} and r_{cv} are the inhibition constants (1/μg) of *P. subcapitata*

Table 1
Combinations of the initial cell densities of *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* used in the laboratory experiments.

Combination	Control		Cu 5 μg/L		Cu 10 μg/L	
	P. subcapitata (10^4 cells/mL)	*C. vulgaris* (10^5 cells/mL)	*P. subcapitata* (10^4 cells/mL)	*C. vulgaris* (10^5 cells/mL)	*P. subcapitata* (10^4 cells/mL)	*C. vulgaris* (10^5 cells/mL)
A	5	2	4	3	4	3
B	3	4	4	8	4	25
C	4	8	12	8	12	8
D	10	4	12	25	12	25
E	7	7	35	8	35	6
F	8	8	35	40	40	32

and *C. vulgaris,* respectively.

To embed the cell density and growth rate-dependent chlorellin product-formation rate into the growth phase model, the Luedeking-Piret equation (Luedeking and Piret, 1959) was employed; this can be expressed as follows:

$$\frac{dp}{dt} = \alpha\frac{dY}{dt} + \beta Y, \tag{4}$$

where Y is the cell density (cells/mL) of *C. vulgaris*, α and β are the growth-related and -unrelated chlorellin formation coefficients (μg/cells), respectively. Yang et al. (2011), who studied the relationship between algal growth and its material formation in detail, reported that the contribution of material formation on the growth-unrelated coefficient was negligible; hence, Eq. (4) can be simplified as follows:

$$\frac{dp}{dt} = \alpha\frac{dY}{dt}, \tag{5}$$

In addition, Franklin et al. (2002) suggested that the initial algal cell density was a determining factor affecting the available copper fraction in the media through algal cellular adsorption, which in turn affects copper toxicity in *P. subcapitata* and *C. vulgaris*. Therefore, once the available copper is taken up by the initial algal cells, it has negligible toxic effect on their growth rate over the exposure duration because of the depletion of the available copper. Thus, the toxic potency of copper can be assumed reciprocal to the initial cell density, and hence Eq. (3) reflecting these assumptions can be rearranged as follows:

$$\begin{aligned}\frac{dX}{dt} &= \mu_{ps}\ \exp(-r_{ps}p)\left(1-\frac{k_{ps}}{t_{ps}X_0 + t_{cv}Y_0}\right)SX - d_{ps}X\\ \frac{dY}{dt} &= \mu_{cv}\ \exp(-r_{cv}p)\left(1-\frac{k_{cv}}{t_{ps}X_0 + t_{cv}Y_0}\right)SY - d_{cv}Y,\end{aligned} \tag{6}$$

where k_{ps} and k_{cv} are the copper toxicity coefficients (cells/mL), t_{ps} and t_{cv} are the specific contribution rates on copper bioavailability, and X_0 and Y_0 are the initial cell densities (cells/mL) of *P. subcapitata* and *C. vulgaris*, respectively.

3.3. Decline phase

To formulate the decline phase of each algal species in the given closed co-culture, it is assumed that the cell density of each algal species will decline and eventually become zero as the habitat quality (S) in the given condition degrades (*i.e.*, S is close to or equal to zero because of nutrient depletion and toxic waste accumulation). Thus, the decline phase model considering the S-dependent- and natural- mortality can be expressed as follows:

$$\begin{aligned}\frac{dX}{dt} &= -a_{ps}\ \frac{X_m - X}{X_m}X - d_{ps}X\\ \frac{dY}{dt} &= -a_{cv}\ \frac{Y_m - Y}{Y_m}Y - d_{cv}Y,\end{aligned} \tag{7}$$

where a_{ps} and a_{cv} are the algal-specific density decrease rates (1/d), and X_m and Y_m are the maximum cell densities (cells/mL) of *P. subcapitata* and *C. vulgaris* under the given closed co-cultivation conditions, respectively.

4. Model calibration, local sensitivity analysis, and simulation

The four ordinary differential equations that describe the time-dependent algal cell density, degradation rate of the habitat quality, and chlorellin formation rate can be described as follows:

$$\begin{aligned}\frac{dX}{dt} &= -d_{ps}X + X\begin{cases}\mu_{ps}\ \exp(-r_{ps}p)\left(1-\frac{k_{ps}}{t_{ps}X_0 + t_{cv}Y_0}\right)S & \text{for } S>0\\ -a_{ps}\frac{X_m - X}{X_m} & \text{otherwise.}\end{cases}\\ \frac{dY}{dt} &= -d_{cv}Y + Y\begin{cases}\mu_{cv}\ \exp(-r_{cv}p)\left(1-\frac{k_{cv}}{t_{ps}X_0 + t_{cv}Y_0}\right)S & \text{for } S>0\\ -a_{cv}\frac{Y_m - Y}{Y_m} & \text{otherwise.}\end{cases}\\ \frac{dS}{dt} &= -m_{ps}X - m_{cv}Y - \begin{cases}\frac{dX}{dt}n_{ps} + \frac{dy}{dt}n_{cv} & \text{for } \frac{dX}{dt}>0 \text{ and } \frac{dY}{dt}>0\\ 0 & \text{otherwise.}\end{cases}\\ \frac{dp}{dt} &= \begin{cases}\alpha\frac{dY}{dt} & \text{for } \frac{dY}{dt}>0\\ 0 & \text{otherwise.}\end{cases}\end{aligned} \tag{8}$$

where X and Y are the cell densities of *P. subcapitata* and *C. vulgaris*, respectively. S is the habitat quality, and p is the chlorellin concentration.

The four differential equations were simulated using the Powersim studio (Powersim software AS, Norway) software employing the Euler integration method with 0.01-d time-step. To calibrate the model parameters, the values of the parameters minimizing the sum of square error (the difference between the observed and predicted cell densities of *P. subcapitata* and *C. vulgaris*) were estimated using the Powersim solver analysis tools.

A local sensitivity analysis was conducted to identify the relative contribution of the input parameters that influence the model output (*e.g.*, maximum algal cell density). The model was implemented by incrementing or decrementing the individual parameter values by 10% with the other parameters fixed, and then calculating the changes in the main model output (Johnston et al., 2014; Gonzalez et al., 2015). The baseline of the sensitivity analysis was the model output calculated by using the estimated parameter values shown in Table 2, the initial *P. subcapitata* density of 2.0×10^5 cells/mL, and the initial *C. vulgaris* density of 2.0×10^6 cells/mL. The parameters a_{ps} and a_{cv}, which represent the specific density decrease rates of *P. subcapitata* and *C. vulgaris*, respectively, were excluded in the sensitivity analysis because these parameters affect algal density only in the decline phase. The parameter sensitivity was expressed as a ratio of the relative change in the maximum algal cell density to the relative change in each parameter value.

Table 2
Estimated model parameters of *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* for the exposed copper concentrations.

Parameter[a]	Copper concentration (μg/L)		
	0	5	10
P. subcapitata			
μ_{ps} (1/d)	0.39	0.30	0.23
m_{ps} (mL/cells)	7.07×10^{-11}	2.17×10^{-8}	1.39×10^{-8}
n_{ps} (mL/cells)	2.53×10^{-7}	6.30×10^{-6}	6.23×10^{-8}
d_{ps} (1/d)	0.007	0.005	0.010
r_{ps} (L/μg)	8.98	8.98	8.98
a_{ps} (1/d)	0.16	0.11	0.11
t_{ps}	–	5.34×10^{-5}	1.68×10^{-4}
k_{ps} (cells/mL)	–	3.33	4.76
C. vulgaris			
μ_{cv} (1/d)	0.41	0.37	0.26
m_{cv} (mL/cells)	3.16×10^{-9}	2.78×10^{-9}	4.73×10^{-11}
n_{cv} (mL/cells)	8.20×10^{-7}	5.30×10^{-8}	2.85×10^{-10}
d_{cv} (1/d)	0.020	0.007	0.008
r_{cv} (L/μg)	1.10	1.10	1.10
a_{cv} (1/d)	0.24	0.12	0.10
α (μg/cells)	1.22×10^{-11}	9.40×10^{-12}	8.41×10^{-12}
t_{cv}	–	3.99×10^{-5}	1.19×10^{-5}
k_{cv} (cells/mL)	–	31.48	47.71

[a] The subscripts 'ps' and 'cv' indicate the parameters related to *Pseudokirchneriella subcapitata* and *Chlorella vulgaris*, respectively.

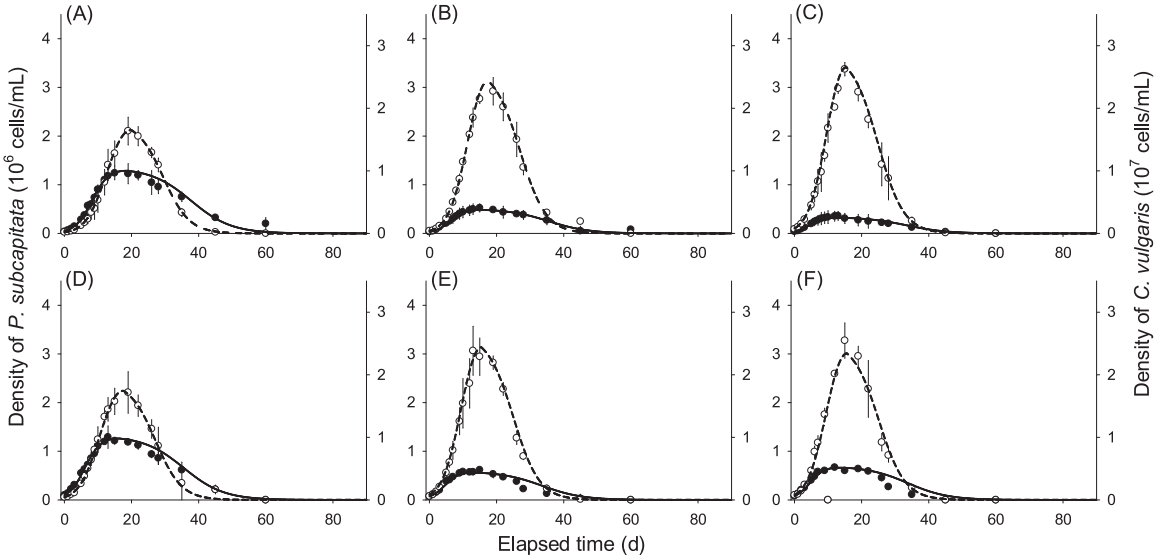

Fig. 2. Predicted and observed algal densities in the control conditions with various initial densities of *Pseudokirchneriella subcapitata* (predicted: solid line, observed: closed circle) and *Chlorella vulgaris* (predicted: dashed line, observed: open circle): (A) 5.0×10^4, 2.0×10^5, (B) 3.0×10^4, 4.0×10^5, (C) 4.0×10^4, 8.0×10^5, (D) 1.0×10^5, 4.0×10^5, (E) 7.0×10^4, 7.0×10^5, and (F) 8.0×10^4, 8.0×10^5 cells/mL. Data are the mean ± SE of four repeated measurements of five repeated treatments. Some small errors are obscured by symbols.

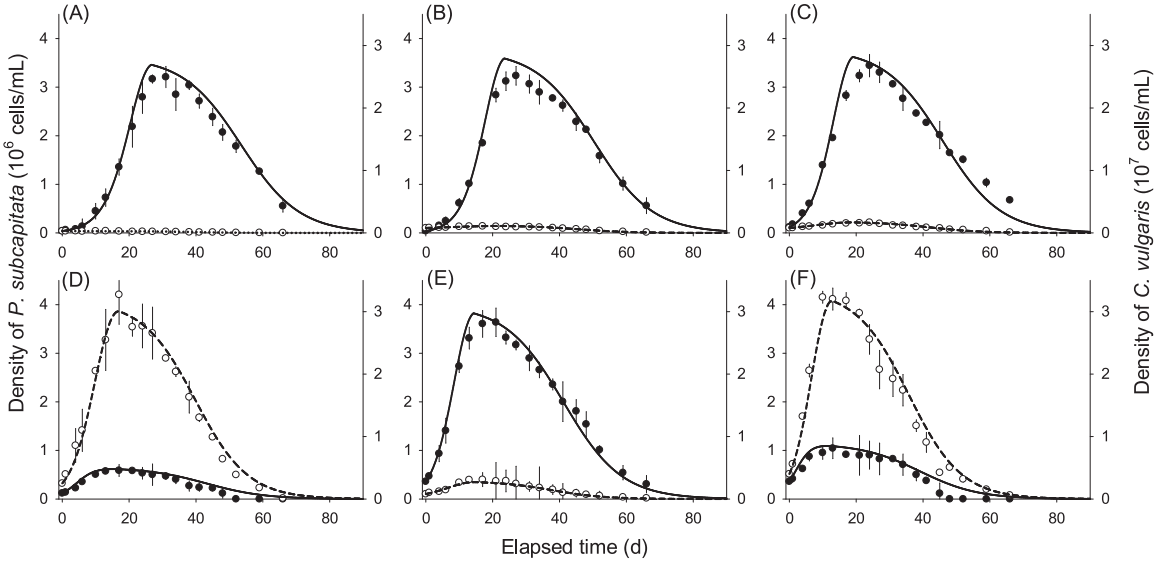

Fig. 3. Predicted and observed algal densities in the 5 μg/L copper-exposed conditions with various initial densities of *Pseudokirchneriella subcapitata* (predicted: solid line, observed: closed circle) and *Chlorella vulgaris* (predicted: dashed line, observed: open circle): (A) 4.0×10^4, 3.0×10^5, (B) 4.0×10^4, 8.0×10^5, (C) 1.2×10^5, 8.0×10^5, (D) 1.2×10^5, 2.5×10^6, (E) 3.5×10^5, 8.0×10^5, and (F) 3.5×10^5, 4.0×10^6 cells/mL. Data are the mean ± SE of four repeated measurements of five repeated treatments. Some small errors are obscured by symbols.

To investigate how the initial cell density and copper exposure level affected the interspecific competition between the two algal species, three indexes, including the competitive dominance, competitive response, and time required to reach the maximum algal density, were simulated by the calibrated model. The competitive dominance was calculated as the log10-transformed ratio of the maximum cell density of *C. vulgaris* to that of *P. subcapitata* (Log_{10} *C. vulgaris/P. subcapitata*) at $S = 0$ in the co-culture. Thus, when the calculated value is a positive or negative, it means a competitive dominance of *C. vulgaris* or *P. subcapitata*, respectively. Furthermore, the competitive response index (referred to herein as a C_{ij} in Eq. (9)) proposed by Fox (2002) was calculated based on each simulated algal cell density. Specifically, the competitive response index in the control was calculated as follows:

$$C_{ij} = (K_i - N_{ij})/K_i, \tag{9}$$

where C_{ij} is the competitive response of species i, N_{ij} is the maximum cell density of species i in the co-culture with a competitor j, and K_i is the maximum cell density of species i in the single culture.

A competitive response was considered a competitive exclusion if $C_{ij} = 1$ or a facilitative effect if $C_{ij} < 0$. Similarly, the effect of copper on the competitive response in the treatments was estimated using a modified version of Eq. (9) as follows:

$$C_{ij\_Cu} = \frac{(K_i - N_{ij_{Cu}})}{K_i}, \tag{10}$$

where C_{ij_Cu} is the competitive responses in the treatments (copper exposed conditions), K_i is the maximum cell density of species i in the single culture, and $N_{ij\ Cu}$ is the maximum cell density of species i with a competitor j in the co-culture under copper exposure. The closer the C_{ij_Cu} value is to 1, the greater the adverse effects of copper on the

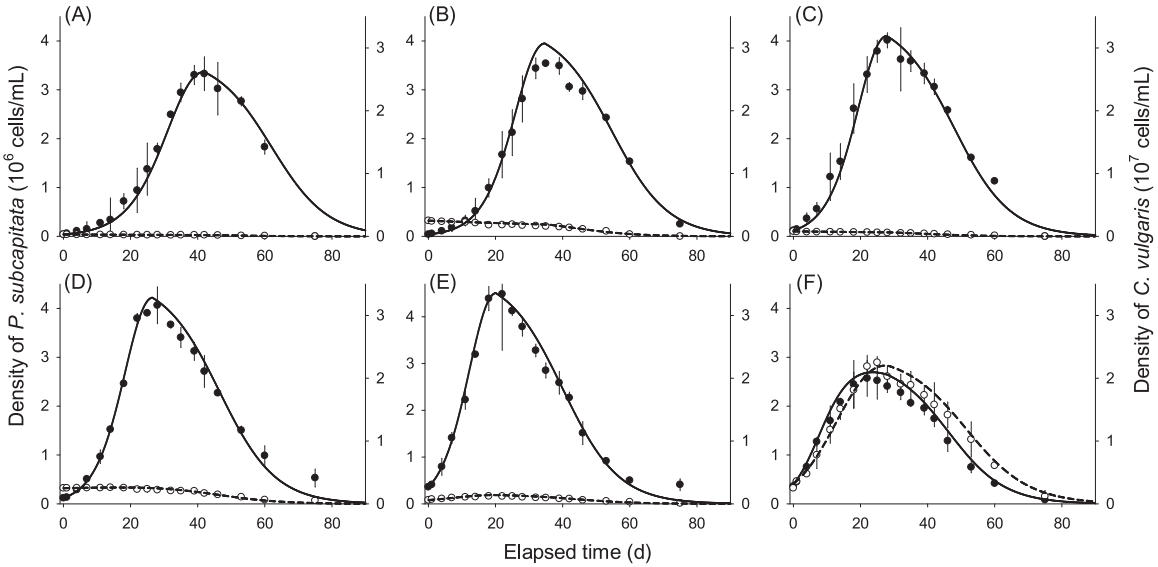

Fig. 4. Predicted and observed algal densities in the 10 μg/L copper-exposed conditions with various initial densities of *Pseudokirchneriella subcapitata* (predicted: solid line, observed: closed circle) and *Chlorella vulgaris* (predicted: dashed line, observed: open circle): (A) 4.0×10^4, 3.0×10^5, (B) 4.0×10^4, 2.5×10^6, (C) 1.2×10^5, 8.0×10^5, (D) 1.2×10^5, 2.5×10^6, (E) 3.5×10^5, 6.0×10^5, and (F) 4.0×10^5, 3.2×10^6 cells/mL. Data are the mean ± SE of four repeated measurements of five repeated treatments. Some small errors are obscured by symbols.

competitive responses of each algal species, whereas the closer this value is to 0, the smaller the adverse effects on the competitive response. For the competitive response calculations, the maximum cell density of each algal species at $S = 0$ as predicted by Eq. (8) was used. The time required to reach the maximum algal density of each algal species was also predicted by Eq. (8).

5. Results

5.1. Experimental results, model calibration, and sensitivity analysis

Experimental observations indicated that the degrees of growth inhibition of both algal species varied with the initial cell density of the competitor species (Figs. 2–4). As shown in Fig. 2, the degree of growth inhibition of *P. subcapitata* was greater than 70% and that of *C. vulgaris* was less than 50% in the co-culture compared with their maximum algal densities in the single culture. It was clear that the higher the initial cell density of the competitor, the higher the growth inhibition of the opponent in the co-cultures. In addition, for both algal species, the higher the initial cell density, the shorter the time required to reach the maximum cell density (Fig. 2D for *P. subcapitata*; Fig. 2C for *C. vulgaris*). Both species began to decline after 20 days with a slight difference in their decline times, and all initial cell density combinations in the control lasted for 45–60 days (Fig. 2). When exposed to copper, the degrees of growth inhibition of both algal species caused by their competitors were different from those observed in the control (Fig. 3). At the copper concentration of 5 μg/L, the maximum cell densities of *C. vulgaris* in the co-cultures were less than 20% of that in the single culture, except for the two combinations wherein the initial cell density of *C. vulgaris* was extremely high. However, the maximum cell density of *P. subcapitata* was 80% of that in the single culture in most initial density combinations, except for two with extremely high initial cell densities. With a copper exposure of 10 μg/L, *P. subcapitata* grew close to the maximum cell density of a single culture in most combinations, but the growth of *C. vulgaris* was completely suppressed, except when in combination with the highest initial cell densities for both algal species (Fig. 4).

A range of model input parameters obtained from the experiments was calibrated before simulating the model, and the calibrated values are listed in Table 2. The relationship between the predicted and observed cell density dynamics of the two algal species was significant, with the coefficients of determination (R^2) being larger than 0.96 for both *P. subcapitata* and *C. vulgaris*, indicating that the developed models were adequately calibrated for the dynamics of both algal species (Figs. 2–4).

The results of the local sensitivity analysis are shown in Table 3. In the control, μ_{ps}, μ_{cv}, and X_0, and μ_{ps}, μ_{cv}, and m_{cv} were found to be relatively important parameters affecting the cell density of *P. subcapitata* and *C. vulgaris*, respectively. Similarly, μ_{ps} and μ_{cv} were found to be the most important parameters in the 5 μg/L copper-exposed group, and the initial cell density of *C. vulgaris* (Y_0) was also an important parameter determining the maximum cell densities of both algal species in the coculture. On the other hand, the parameters related to the effect of copper on *P. subcapitata* and *C. vulgaris* (t_{ps}; and k_{cv} and t_{cv}, respectively) and the parameters related to the initial cell densities of both species (X_0 and Y_0) had the greatest effect on cell density in the 10 μg/L copper-exposed group.

5.2. Simulation results on competitive dominance

When competitive dominance was calculated based on the simulated maximum cell density across a range of initial cell densities for both species, *C. vulgaris* showed competitive dominance over *P. subcapitata*, regardless of the initial cell densities of both species (Fig. 5A). However, it was also true that the higher the initial density of *P. subcapitata*, the greater the extent that the competitive dominance of *C. vulgaris* over *P. subcapitata* was offset. The simulation results for the 5 μg/L copper exposure showed that *C. vulgaris* dominated the co-culture when the initial cell density of *C. vulgaris* was higher than 15×10^5 cells/mL, regardless of the initial cell density of *P. subcapitata*; however, *C. vulgaris* became less dominant when its initial cell density was less than 15×10^5 cells/mL (Fig. 5B). However, unlike the control and 5 μg/L copper-exposed groups, the competitive dominance of *C. vulgaris* over *P. subcapitata* was completely reversed at an exposure to 10 μg/L of copper. As seen in Fig. 5C, the competitive dominance of *C. vulgaris* tended to be relatively low even at high initial cell densities of *C. vulgaris* and low initial cell densities of *P. subcapitata*. The likelihood of the competitive dominance of *C. vulgaris* over *P. subcapitata* was achieved only when the initial cell densities of both algal species were high. These results suggested that high initial cell densities of both algal

Table 3
Sensitivity of model parameters in each experimental condition. The absolute values of sensitivity larger than 1.0 and 0.5 are indicated by dark gray and light gray shading, respectively.

		Control		Cu 5 μg/L		Cu 10 μg/L	
Parameter[1)]	Tested (%)	*X*	*Y*	*X*	*Y*	*X*	*Y*
μ_{ps}	+10	1.523	–0.475	1.888	–0.767	0.786	–0.413
	–10	–1.333	0.428	–1.660	0.757	–0.850	0.519
m_{ps}	+10	<0.001	<0.001	–0.088	–0.338	–0.557	–0.109
	–10	<0.001	<0.001	0.086	0.360	0.635	0.118
n_{ps}	+10	–0.021	–0.240	–0.030	–0.112	–0.309	–0.067
	–10	0.020	0.244	0.029	0.114	0.332	0.070
d_{ps}	+10	–0.074	0.006	–0.078	0.018	–0.120	0.022
	–10	0.076	–0.006	0.079	–0.018	0.119	–0.022
r_{ps}	+10	–0.627	0.184	–0.655	0.208	–0.080	0.024
	–10	0.745	–0.210	0.736	–0.219	0.080	–0.023
t_{ps}	+10	–	–	–0.086	0.102	–0.274	2.210
	–10	–	–	0.089	–0.104	0.253	–1.956
k_{ps}	+10	–	–	–0.068	0.029	–0.074	0.042
	–10	–	–	0.068	–0.029	0.074	–0.041
X_0	+10	0.977	–0.241	0.780	–0.345	–0.173	1.923
	–10	–0.982	0.245	–0.813	0.374	0.181	–1.863
μ_{cv}	+10	–1.170	1.020	–1.347	1.687	–0.110	0.764
	–10	1.612	–1.155	1.697	–1.665	0.103	–0.701
m_{cv}	+10	–0.032	–0.461	–0.124	–0.473	–0.004	–0.001
	–10	0.029	0.521	0.123	0.539	0.004	0.001
n_{cv}	+10	–0.019	–0.249	–0.003	–0.013	–0.001	<0.001
	–10	0.018	0.262	0.003	0.014	0.001	<0.001
d_{cv}	+10	0.042	–0.153	0.028	–0.084	0.005	–0.177
	–10	–0.042	0.153	–0.028	0.085	–0.005	0.182
r_{cv}	+10	0.070	–0.148	0.058	–0.121	0.001	–0.006
	–10	–0.070	0.153	–0.058	0.125	–0.001	0.006
t_{cv}	+10	–	–	–0.572	0.706	–0.192	1.540
	–10	–	–	0.758	–0.838	0.182	–1.412
k_{cv}	+10	–	–	0.859	–0.895	0.453	–2.931
	–10	–	–	–0.759	0.901	–0.615	4.518
Y_0	+10	–0.605	0.284	–1.226	1.248	–0.299	2.729
	–10	0.729	–0.323	1.659	–1.439	0.244	–2.278
α	+10	–0.567	0.033	–0.601	0.083	–0.079	0.018
	–10	0.663	–0.060	0.674	–0.099	0.079	–0.018

[a]The subscripts 'ps' and 'cv' indicate the parameters related to *Pseudokirchneriella subcapitata* and *Chlorella vulgaris*, respectively.

species could mitigate copper toxicity, specifically in *C. vulgaris*.

5.3. Simulation results on competitive response

The competitive response reflecting the extent to which each algal species was affected in the presence of the competitor is shown in Fig. 6A and B. The competitive response of each species tended to be close to zero as the initial cell density increased in the control (Fig. 6A and B), indicating that the higher the initial cell density of a species in the co-culture, the greater the probability of its dominating the competitor. The simulated results also reflected that in the 5 μg/L copper exposed group, the competitive response of *C. vulgaris* was weaker than that of the control as the initial density of *P. subcapitata* increased, reflecting a decrease in contour line values (Fig. 6D), while the opposite was observed for *P. subcapitata* (Fig. 6C). *P. subcapitata* was able to grow under copper exposure, even with a low initial cell density in the co-culture and could achieve the same cell density as in the single culture, regardless of its own initial density as well as that of a competitor, even at 10 μg/L copper exposure (Fig. 6E). The growth inhibition of *C. vulgaris* in co-culture was more severe than that in the single culture for most initial cell densities of both species (Fig. 6F).

5.4. Simulation results on the time required to reach maximum algal density

The simulation results showed that the time required to reach the maximum algal density increases from less than 10 d to more than 50 d as the copper concentration increases (Fig. 7). In detail, the time required to reach the maximum density of *P. subcapitata* increased under

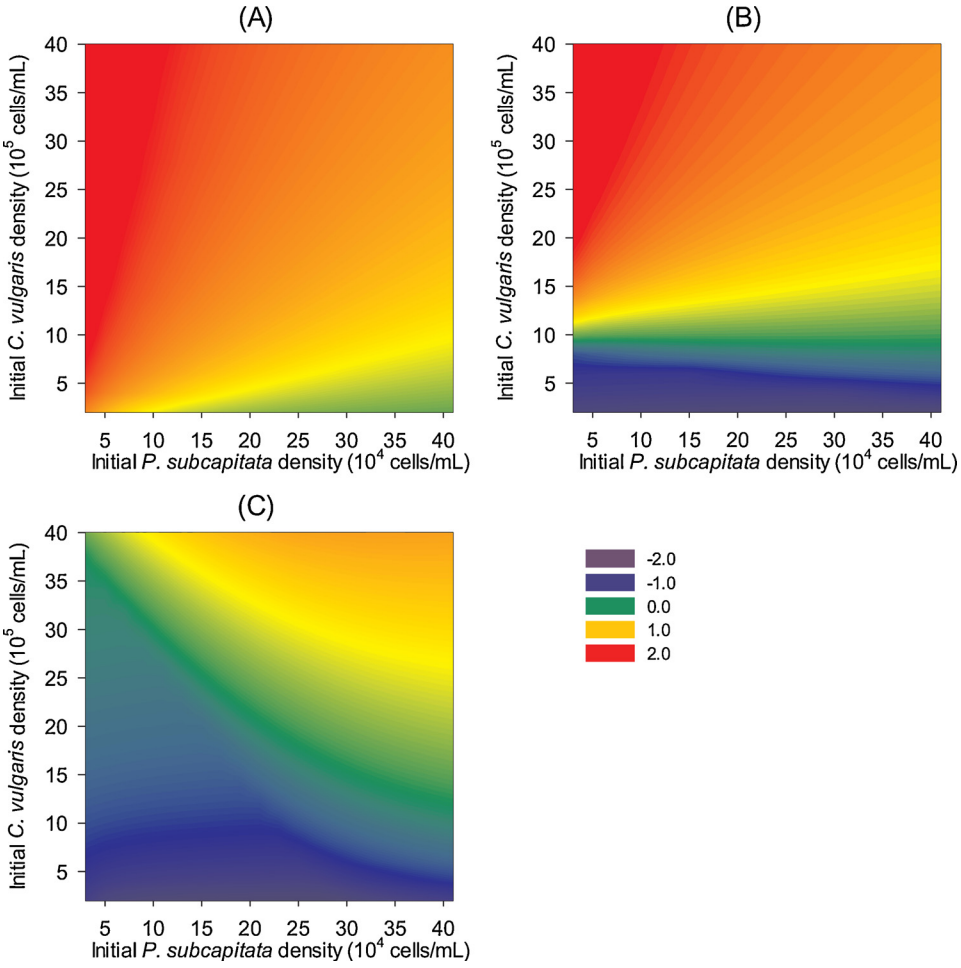

Fig. 5. Filled contour plots for the competitive dominance of *Chlorella vulgaris* over *Pseudokirchneriella subcapitata* in co-culture under the absence (A), 5 μg/L (B), and 10 μg/L (C) of copper, using the simulated data under a range of initial algal cell densities of the two species. The values between –2 (purple color) and 2 (red color) indicate the simulated maximum algal density ratio of *C. vulgaris* to that of *P. subcapitata* from 0.01 to 100 (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

the conditions of relatively low initial densities of both algal species and 5 μg/L of copper exposure (Fig. 7A and C). At 10 μg/L of copper exposure, the initial density of *P. subcapitata* directly affected the time required for it to reach its maximum density (Fig. 7E). On the other hand, the time for *C. vulgaris* to reach its maximum density at 5 μg/L of copper exposure showed a pattern similar to that of *P. subcapitata* (Fig. 7C and D), but the initial density of *C. vulgaris* had a considerable influence on the required time in the control and 10 μg/L copper exposure conditions (Fig. 7B and F). The purple areas in Fig. 7D and F show short times to reach maximum *C. vulgaris* density, but, in fact, this result was due to its suppressed growth and low maximum density as shown in the same area in Fig. 6D and F. Overall, the initial density of each algal species and the level of copper exposure dramatically influenced the time required to achieve maximum algal density in a co-culture condition.

6. Discussion

In this study, both experimental and mathematical modeling approaches were employed to investigate how various combinations of the initial cell densities of *P. subcapitata* and *C. vulgaris* and copper exposure conditions affected interspecific competition between these species. The results showed that the degree of growth inhibition and competition in the co-cultures varied with the competitor's initial cell density and copper exposure level, which in turn resulted in shifts in the competitive dominance of each algal species in the given co-cultures. Furthermore, the simulation results showed that the initial density of the two competing species had different effects on the competitive response and the time required to reach the maximum algal density depending on the copper exposure level. These results indicate that the result of interspecific competition between algal species in natural ecosystems is not simply determined by the superiority or inferiority of the species, and will show complex patterns due to environmental conditions, including time and space.

Several studies have demonstrated that the ecological phenomena observed in freshwater ecosystems, such as algal blooms and the dominance of a specific algal species, could be explained by understanding the competition between algal species (Tameishi et al., 2009; Qiu et al., 2011). However, in their study, the effect of initial algal density on the interspecific competition showed different results (*e.g.*, Qiu et al., 2011: initial density-dependency; Tameishi et al., 2009: initial density-independency). These conflicting results between studies might be due in part to the differences between the tested species, but might also be due to the differences in the initial cell density of the species tested. Our study provided a detailed description of how various initial cell density combinations of *P. subcapitata* and *C. vulgaris* affect the interspecific competition through numerical simulations in a range of initial algal cell densities. Our study clearly showed that the initial density of each algal species is a driving factor determining the growth of the competing species in the absence of copper exposure; however, *C. vulgaris* could hold a dominant position in the co-culture, regardless of the initial cell densities of both species. This was because *C. vulgaris*,

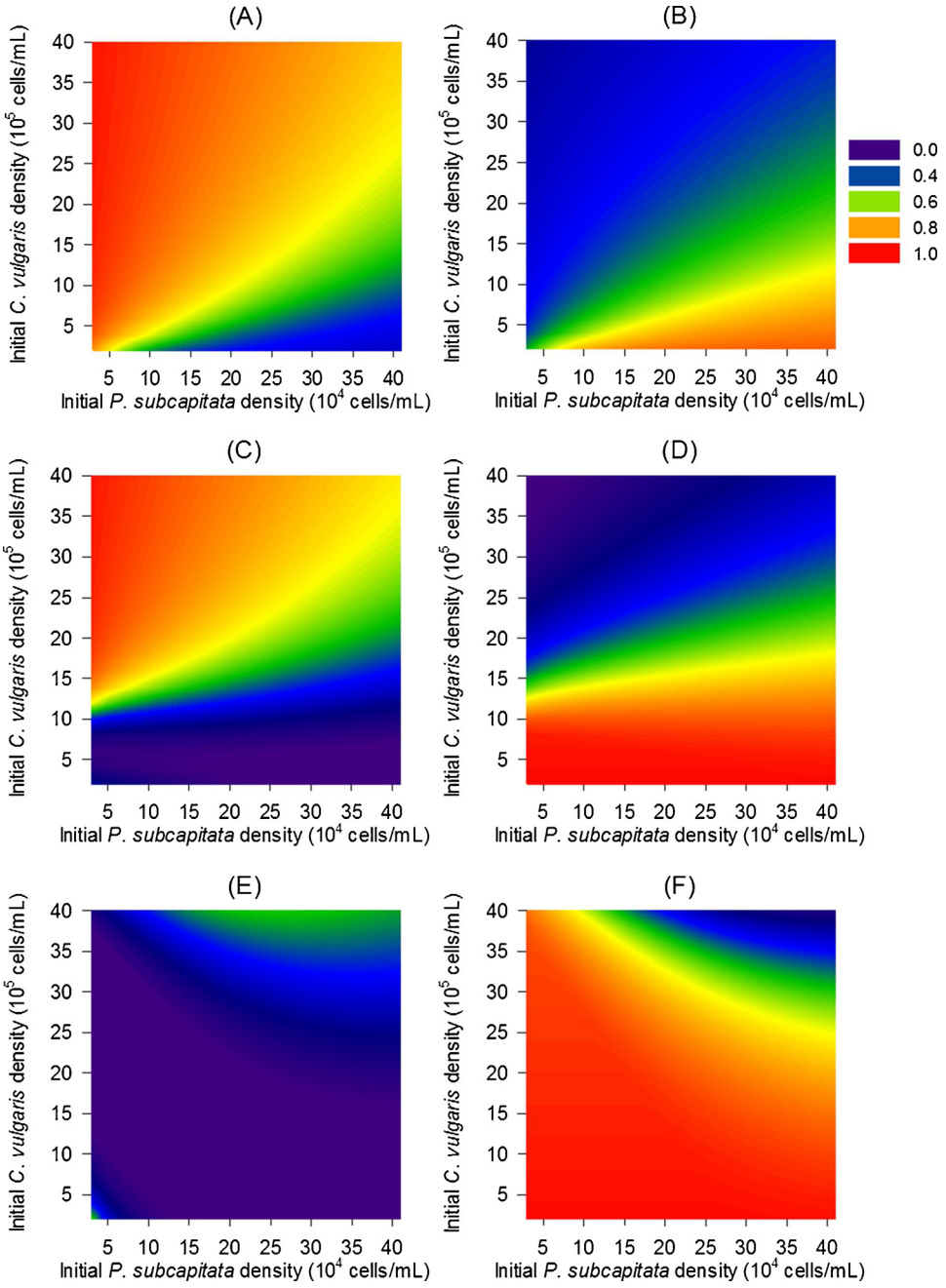

Fig. 6. Filled contour plots for the calculated competitive responses of *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* in co-culture under the absence (A and B), 5 μg/L (C and D), and 10 μg/L (E and F) of copper-exposure, using the simulated algal density under a range of initial algal cell densities of the two species. The values of 0 (purple color) and 1 (red color) indicate that the algal species is not negatively affected by competition and excluded from competition, respectively (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

which is known to produce allelochemicals, could regulate the growth of *P. subcapitata* in the co-culture, resulting in its competitive advantage over *P. subcapitata*. This result is in line with the studies conducted by Tillmann et al. (2007) and Granéli et al. (2008), who reported that the higher initial cell density of the algal species producing allelochemicals, the greater the influence on the competing species.

In the present study, we also examined how various combinations of the initial cell densities of *P. subcapitata* and *C. vulgaris* and copper exposure levels affect interspecific competition. Although the inhibitory effect of *C. vulgaris* on *P. subcapitata* was strong enough to reduce the growth of *P. subcapitata* in the absence of copper exposure, the outcome in the co-culture highly varied with the initial cell densities of the competitor and the copper concentrations to which it is exposed. While the influx of contaminants into aquatic ecosystems is common, little research has been done on the impact of contaminants on the intraspecific or interspecific competition among algal species. Lürling and Roessink (2006) reported that an exposure to the herbicide metribuzin could reverse the interspecific competition between *Microcystis aeruginosa* and *Scenedesmus obliquus*, suggesting that the difference in sensitivity to contaminants could lead to a change in the algal species dominating aquatic ecosystems. Similarly, our results showed that competitive dominance could be reversed when the algal species are exposed to copper, especially to a concentration of 10 μg/L copper. Although the mechanisms underlying the impact of copper exposure on chlorellin production by *C. vulgaris* could not be fully identified in this study, the initial cell density and copper exposure levels influenced the

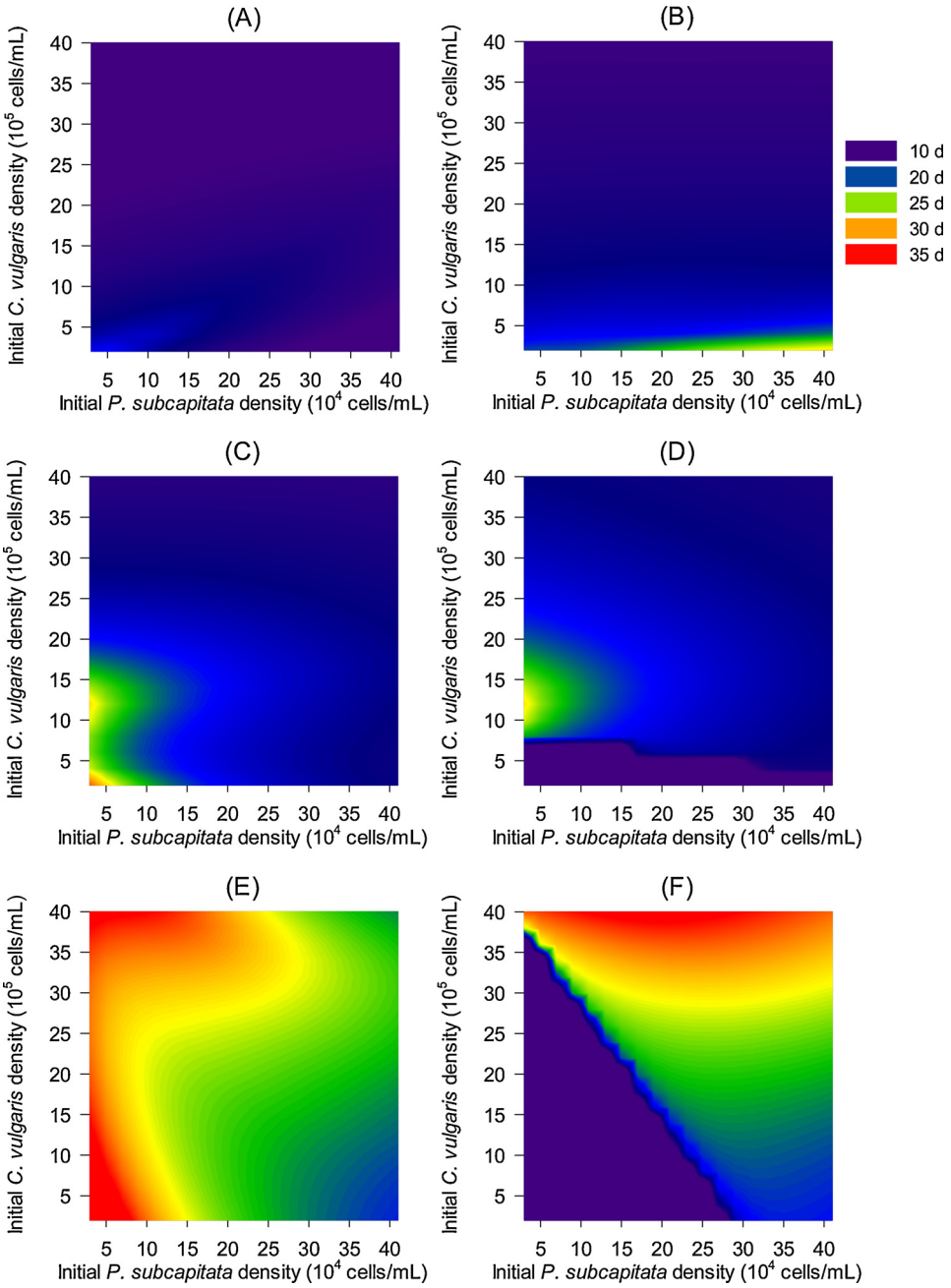

Fig. 7. Filled contour plots for the predicted time to reach the maximum algal cell density for *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* in co-cultures under the absence (A and B), 5 μg/L (C and D), and 10 μg/L (E and F) of copper-exposure through simulation under a range of initial algal cell densities of the two species. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

interspecific competition by changing the algal growth and the amount of producing chlorellin. Especially at 10 μg/L of copper exposure, the copper-sensitive *C. vulgaris* was excluded from the competition, providing the competitive advantage to the copper-resistant *P. subcapitata*. However, this copper exposure-induced competitive advantage of *P. subcapitata* weakens as the initial density of both algal species increases. This result indicates that increased initial cell densities of both algal species can mitigate copper toxicity, specifically in *C. vulgaris*.

In addition to the factors considered in this study, a wide variety of factors can influence the interspecific competition among algal species. As a result, predicting the dominant algal species will be complicated by the environmental conditions varied in time and space scales (*e.g.*, seasonal environmental conditions, spatial locations and size of habitats). The predator population that feeds on algae will be affected by the dominant algal species, and the community structure of the freshwater ecosystem will change over time. This near-chaotic complexity of the algal community known as 'the paradox of the plankton' has been proposed by Hutchinson (1961); this is one of the abstruse questions in ecology determining why plankton communities can never be at equilibrium in real ecosystems. Although many researchers have accepted the unpredictable complexity of the algal community, the mathematical model can be a useful tool to gain a mechanistic understanding for connecting the observations from the individual level to ecosystems (Zhao et al., 2008; Sommer et al., 2012).

The present study, to our knowledge, is the first modeling approach to elucidate the influence of initial cell density and copper exposure on the interspecific competition between two algae species. Previously, Fergola et al. (2007) and DellaGreca et al. (2010) showed the

mathematical models mechanistically describing the role of allelochemicals in the interspecific competition between *P. subcapitata* and *C. vulgaris*. Our study has further enhanced their findings by incorporating the effects of initial cell density and copper exposure into the new model structure. In addition, the model was verified using the experimental data for a sufficient number of initial cell density combinations. Although the model was applied to *P. subcapitata* and *C. vulgaris*, it will be possible to study interspecific competition among other algae species using the proposed model structure.

In this study, the experimental observations and mathematical modeling were performed to explain the essential mechanism, rather than the quantitative realism, of the algal competition. The proposed mathematical model is too simple to describe algal dynamics in aquatic ecosystems, but is useful for simulating the effects of a wide range of factors on the interspecific competition that are difficult to observe experimentally. The simulation results showed that the changes in initial density and copper exposure levels greatly influenced algal competition in terms of competitive dominance, competitive response, and time required to reach maximum algal density and clearly explained how much these factors affected the interspecific competition of each algal species. Given that initial density and copper exposure levels have a great impact on the interspecific competition between two algal species, as shown in this study, further studies of the impact of other factors are needed to understand interspecific competition in aquatic ecosystems.

Acknowledgments

This subject is supported by Korea Ministry of Environment (MOE) as “The Chemical Accident Prevention Technology Development Project.” (2016001970003) and by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2017R1A6A3A11035451).

References

Carusela, M.F., Momo, F.R., Romanelli, L., 2009. Competition, predation and coexistence in a three trophic system. Ecol. Model. 220, 2349–2352.
Chakraborty, S., Roy, S., Chattopadhyay, J., 2008. Nutrient-limited toxin production and the dynamics of two phytoplankton in culture media: a mathematical model. Ecol. Model. 213, 191–201.
Chase, J.M., Abrams, P.A., Grover, J.P., Diehl, S., Chesson, P., Holt, R.D., Richards, S.A., Nisbet, R.M., Case, T.J., 2002. The interaction between predation and competition: a review and synthesis. Ecol. Lett. 5, 302–315.
Connell, J.H., 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. Am. Nat. 122, 661–696.
DellaGreca, M., Zarrelli, A., Fergola, P., Cerasuolo, M., Pollio, A., Pinto, G., 2010. Fatty acids released by *Chlorella vulgaris* and their role in interference with *Pseudokirchneriella subcapitata*: experiments and modelling. J. Chem. Ecol. 36, 339–349.
EPA, 2002. Method 1003.0: Green Alga Selenastrum capricornutum, Growth Test; Chronic Toxicity.
Fergola, P., Cerasuolo, M., Pollio, A., Pinto, G., DellaGreca, M., 2007. Allelopathy and competition between *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*: experiments and mathematical model. Ecol. Model. 208, 205–214.
Flemming, C.A., Trevors, J.T., 1989. Copper toxicity and chemistry in the environment: a review. Water Air Soil Pollut. 44, 143–158.
Fox, J.W., 2002. Testing a simple rule of dominance in resource competition. Am. Nat. 159, 305–319.
Franklin, N.M., Stauber, J.L., Apte, S.C., Lim, R.P., 2002. Effect of initial cell density on the bioavailability and toxicity of copper in microalgae bioassays. Environ. Toxicol. Chem. 21, 742–751.
Garvey, J.E., Stein, R.A., Thomas, H.M., 1994. Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. Ecology 75, 532–547.
Gonzalez, D., Cabral, J.A., Torres, L., Santos, M., 2015. A cohort-based modelling approach for managing olive moth *Prays oleae* (Bernard, 1788) populations in olive orchards. Ecol. Model. 296, 46–56.
Granéli, E., Weberg, M., Salomon, P.S., 2008. Harmful algal blooms of allelopathic microalgal species: the role of eutrophication. Harmful Algae 8, 94–102.
Hu, S., Zhang, D.Y., 1993. The effects of initial population density on the competition for limiting nutrients in two freshwater algae. Oecologia 96, 569–574.
Hutchinson, G.E., 1961. The paradox of the plankton. Am. Nat. 75, 406–418.
Hulot, F.D., Huisman, J., 2004. Allelopathic interactions between phytoplankton species: the roles of heterotrophic bacteria and mixing intensity. Limnol. Oceanogr. 49, 1424–1434.
Johnston, A.S.A., Hodson, M.E., Thorbek, P., Alvarez, T., Sibly, R.M., 2014. An energy budget agent-based model of earthworm populations and its application to study the effects of pesticides. Ecol. Model. 280, 5–17.
Kuwata, A., Miyazaki, T., 2000. Effects of ammonium supply rates on competition between *Microcystis novacekii* (Cyanobacteria) and *Scenedesmus quadricauda* (Chlorophyta): simulation study. Ecol. Model. 135, 81–87.
Luedeking, R., Piret, E.L., 1959. A kinetic study of the lactic acid fermentation: batch process at controlled pH. Biotechnol. Bioeng. 1, 363–394.
Lürling, M., Roessink, I., 2006. On the way to cyanobacterial blooms: impact of the herbicide metribuzin on the competition between a green alga (*Scenedesmus*) and a cyanobacterium (*Microcystis*). Chemosphere 65, 618–626.
Machado, M.D., Lopes, A.R., Soares, E.V., 2015. Responses of the alga *Pseudokirchneriella subcapitata* to long-term exposure to metal stress. J. Hazard. Mater. 296, 82–92.
Qiu, X., Yamasaki, Y., Shimasaki, Y., Gunjikake, H., Matsubara, T., Nagasoe, S., Etoh, T., Matsui, S., Honjo, T., Oshima, Y., 2011. Growth interactions between the raphidophyte *Chattonella antiqua* and the dinoflagellate *Akashiwo sanguinea*. Harmful Algae 11, 81–87.
Silva, A., Figueiredo, S.A., Sales, M.G., Delerue-Matos, C., 2009. Ecotoxicity tests using the green algae *Chlorella vulgaris*-a useful tool in hazardous effluents management. J. Hazard. Mater. 167, 179–185.
Sommer, U., Adrian, R., De Senerpont Domis, L., Elser, J.J., Gaedke, U., Ibelings, B., Jeppesen, E., Lürling, M., Molinero, J.C., Mooij, W.M., van Donk, E., Winder, M., 2012. Beyond the plankton ecology group (PEG) model: mechanisms driving plankton succession. Annu. Rev. Ecol. Evol. Syst. 43, 429–448.
Stoiber, T.L., Shafer, M.M., Armstrong, D.E., 2012. Relationships between surface-bound and internalized copper and cadmium and toxicity in *Chlamydomonas reinhardtii*. Environ. Toxicol. Chem. 31, 324–335.
Tameishi, M., Yamasaki, Y., Nagasoe, S., Shimasaki, Y., Oshima, Y., Honjo, T., 2009. Allelopathic effects of the dinophyte *Prorocentrum minimum* on the growth of the bacillariophyte *Skeletonema costatum*. Harmful Algae 8, 421–429.
Tillmann, U., John, U., Cembella, A., 2007. On the allelochemical potency of the marine dinoflagellate *Alexandrium ostenfeldii* against heterotrophic and autotrophic protists. J. Plankton Res. 29, 527–543.
Uchida, T., Toda, S., Matsuyama, Y., Yamaguchi, M., Kotani, Y., Honjo, T., 1999. Interactions between the red tide dinoflagellates *Heterocapsa circularisquama* and *Gymnodinium mikimotoi* in laboratory culture. J. Exp. Mar. Biol. Ecol. 241, 285–299.
Yamasaki, Y., Nagasoe, S., Matsubara, T., Shikata, T., Shimasaki, Y., Oshima, Y., Honjo, T., 2007. Allelopathic interactions between the bacillariophyte *Skeletonema costatum* and the raphidophyte *Heterosigma akashiwo*. Mar. Ecol. Prog. Ser. 339, 83–92.
Yang, J., Rasa, E., Tantayotai, P., Scow, K.M., Yuan, H., Hristova, K.R., 2011. Mathematical model of *Chlorella minutissima* UTEX2341 growth and lipid production under photoheterotrophic fermentation conditions. Bioresour. Technol. 102, 3077–3082.
Zhao, J., Ramin, M., Cheng, V., Arhonditsis, G.B., 2008. Competition patterns among phytoplankton functional groups: how useful are the complex mathematical models? Acta Oecol. 33, 324–344.